

CM WHAT IS CLAIMED IS:

1. A method for preparing a binding polypeptide which consists essentially of the amino acid sequence of at least a portion of the variable region of a light or heavy chain of an immunoglobulin specific for a predetermined ligand, said amino acid sequence having substantially the same binding specificity of the analogous chain,

said method comprising:

preparing ds cDNA encoding at least one of said light or heavy chains from an mRNA coding for said chain;

removing nucleotide sequences from said ds cDNA superfluous to said variable region while providing for initiation and termination codons at the 5'- and 3'-termini respectively of the DNA sequence to provide tailored ds cDNA encoding said variable region;

inserting said tailored ds cDNA into an expression vector for expression of said ds cDNA and transforming a host for said expression vector with said ds cDNA containing expression vector;

growing said transformed host, whereby said binding polypeptide of one of said light and heavy chains is expressed; and

isolating said binding polypeptide.

2. A method according to Claim 1, wherein said immunoglobulin is IgG.

3. A method according to Claim 1, wherein said initiation and termination codons are provided by in vitro mutagenesis.

4. A method according to Claim 1, including the additional step of prior to inserting, replacing at least one nucleotide in said ds cDNA to change a codon to encode for a different amino acid.

5. A method according to any of Claims 1, 2, 3, or 4 wherein said binding polypeptide is substantially the sequence of said light chain.

6. A method according to any of Claims 1, 2, 3 or 4 wherein said binding polypeptide is substantially the sequence of said heavy chain.

7. A method for preparing a binding protein ("rFv") comprising two polypeptide chains each of from about 95 to 125 amino acids, which complex together to bind to a predetermined ligand,

said method comprising:

preparing ds cDNA coding for the light and heavy chains of an immunoglobulin, said chains complexing together to bind to a predetermined ligand, wherein each of said chains is comprised of a constant region and a variable region, said variable regions being of from about 95 to 125 amino acids; said preparing comprising isolating mRNA coding for said chains, reverse transcribing said mRNA to produce ss cDNA, synthesizing a strand complementary to said ss cDNA by means of DNA polymerase to produce ds cDNA having a coding strand coding for said light or heavy chain, said coding strands including sequences coding for leader sequence, variable region and constant region in the 5'-3' direction of said coding strand, and cloning said ds cDNA;

removing at least a portion of the regions flanking said variable regions of said light or heavy chains, said removing comprising providing a coding or non-coding ss cDNA strand from said cloned ds cDNA, followed by the following sequences of steps:

(1) hybridizing to the non-coding strand a first oligonucleotide primer having an initiation codon for defining the initiation site for expression of said variable regions, wherein said first oligonucleotide is complementary to the sequence coding for the N-terminus of the leader region or partially complementary to the DNA sequence coding for the juncture of the leader region and variable region,

having a non-complementary initiation codon about at said juncture and enzymatically treating the resulting duplex to elongate the primer in the 5'-3' direction of said first oligonucleotide complementary to said non-coding ss cDNA, while digesting said non-coding ss cDNA in the 3'-5' direction up to the sequence complementary to said first oligonucleotide primer, and cloning the resulting N-terminus defined ds cDNA;

(2) hybridizing to the coding strand a second oligonucleotide primer having a stop codon and complementary to the sequence at about the juncture of said variable region and said constant region, wherein said stop codon non-complementary to said sequence is at said juncture for introducing a stop codon at the terminus of said variable region, and enzymatically treating the resulting duplex to elongate said primer in the 5'-3' direction of said second oligonucleotide primer complementary to said coding strand and digesting said coding ss cDNA in the 3'-5' direction up to the sequence complementary to said second oligonucleotide primer and cloning the resulting C-terminus tailored ds cDNA,

whereby N- and C-terminus tailored ds cDNA is obtained coding for the variable region of the light or heavy chain free of the constant region of said immunoglobulin;

inserting N- and C-terminus tailored ds cDNA into an expression vector with said coding sequence in proper relationship with transcriptional and translational regulatory signals;

transforming a host with said expression vector and growing said host, whereby the light or heavy variable region polypeptides are expressed; and

combining said light and heavy region polypeptides to form said rFv.

8. A method according to Claim 7, wherein said first oligonucleotide primer homoduplexes with said non-coding strand at the N-terminus of said leader sequence and

said growing includes secreting by said host of said variable region polypeptides free of the leader sequence.

5 9. A method according to Claim 7, wherein said first oligonucleotide primer hybridizes to about the juncture between said leader sequence and said variable sequence to introduce an initiation codon at the N-terminus of the DNA sequence coding for said variable region.

10 10. A method according to any of Claims 7, 8 or 9, including the additional step of prior to said inserting, ligating unique restriction linkers to said N- and C-terminus tailored ds cDNA and enzymatically cleaving said linkers to provide cohesive termini.

15 11. A method according to any of Claims 7, 8 or 9, wherein said cloning is repeated, wherein either of said oligonucleotides are only partially complementary to said strand of said ds cDNA.

12. A method according to Claim 7, wherein said host is a bacterium.

20 13. A method according to Claim 7, wherein said host is a yeast.

14. A method according to Claim 7, wherein said immunoglobulin is IgG.

15. A method according to Claim 7, wherein said variable region of said heavy chain includes the D sequence.

25 16. A method according to any of Claims 7, 8 or 9, wherein said ligand is an enzyme.

17. A method according to any of Claims 7, 8 or 9, wherein said ligand is a surface protein.

18. A method for preparing a binding protein ("rFv") comprising two polypeptide chains, a light chain variable region of from about 95 to 115 amino acids and a heavy chain variable region of from about 110 to 125 amino acids, which chains complex together to bind with high affinity to a predetermined ligand,

said method comprising:

preparing ds cDNA coding for the light and heavy chains of IgG specific for said predetermined ligand, wherein each of said chains is comprised of a variable region and a constant region, said preparing comprising isolating mRNA from cloned cells producing said IgG coding for said light and heavy chains, reverse transcribing said mRNA to produce complementary ss cDNA, synthesizing a strand complementary to said ss cDNA by means of DNA polymerase to produce ds cDNA having a coding strand coding for said light or heavy chains, wherein said coding strand includes leader, variable region and constant region sequences in the 5'-3' direction, and cloning said ds cDNA;

removing regions flanking the sequences coding for said variable regions, said removing comprising preparing partially complementary first and second oligonucleotide primers and heteroduplexing said primers with the complementary strand of said ds cDNA for the purpose of introducing start and stop codons, respectively, at the N- and C-termini of said variable regions, followed by the following sequences of steps:

(1) hybridizing to the non-coding strand of said ds cDNA said first oligonucleotide primer having DNA sequences complementary to the sequence at about the juncture between the leader sequence and the variable region sequence, enzymatically treating the resulting duplex to replicate the non-coding ss cDNA in the 5'-3' direction of said first oligonucleotide primer, while digesting said non-coding ss cDNA in the 3'-5' direction up to the sequence complementary to said first oligonucleotide primer, and cloning the resulting N-terminus tailored ds cDNA;

(2) hybridizing to the coding strand said second oligonucleotide primer complementary to the DNA sequence at about the juncture between the sequences defining the variable region and the constant region to provide a stop codon at the terminus of the variable region, enzymatically treating the resulting duplex to produce a DNA strand complementary to the coding ss cDNA in the 5'-3' direction of said second oligonucleotide primer and digesting said coding ss cDNA in the 3'-5' direction up to the sequence complementary to said second oligonucleotide primer sequence, and cloning the resulting C-terminus tailored ds cDNA,

whereby N- and C-terminus tailored ds cDNA is obtained coding for the variable region of the light or heavy chain free of the constant region and leader sequences;

inserting said N- and C-terminus tailored ds cDNA into an expression vector with said coding sequence in proper relationship with transcriptional and translational regulatory signals;

transforming a host with said expression vector and growing said host, whereby the light or heavy variable region polypeptides are expressed; and

combining said light and heavy region polypeptides to form said rFv.

19. A method according to Claim 18, wherein said cloning after each hybridizing step includes the additional step of selecting clones having said first or second oligonucleotide sequence, isolating the DNA containing said ds cDNA and recloning said ds cDNA.

20. A specific binding composition, ^("rFv") comprising two polypeptide chains having substantially the same amino acid sequence of at least a portion of the variable region of an immunoglobulin, said immunoglobulin having binding specificity to a predetermined ligand, wherein said polypeptide chains are prepared by translation of a DNA sequence coding for the variable region free of the constant region, and

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wherein said two polypeptide chains combine to form ^{the} a complex having a high affinity and specificity for said predetermined ligand.

22. A composition according to Claim 20, wherein said two polypeptide chains are the light chain of from about 95 to 115 amino acids and the heavy chain of from about 110 to 125 amino acids, wherein said heavy chain includes the D region.

23. A composition according to any ^{ONE} of Claims 20 or 21, wherein each of said chains includes at least two cysteines separated by from about 60 to 70 amino acids and joined together through a disulfide link ~~to define a domain~~.

24. A composition according to any ^{ONE} of Claims 20 or 21, wherein said rFv is labeled with a functionality capable of producing a detectable signal.

25. A composition according to any ^{ONE} of Claims 20 or 21, wherein said rFv is labeled with a cytotoxic agent.

26. A composition according to Claim 24, wherein said cytotoxic agent is a radionuclide.

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